an IAV infection and well above the 1:40 HAI titer associated with protection (43) suggesting that HA-specific antibodies are equal.

Based on the observed protective ability of Trm against IAV, it has recently been suggested that a "universal" vaccine against IAV should induce such T cell responses in order to offer the greatest level of protection. Importantly, analysis of the lungs after IAV-nanovax vaccination found the presence of IAV-specific CD4 and CD8 T cells. These IAV-specific CD4 and CD8 T cells were within the lung parenchyma based on CD45 i.v.Ab exclusion staining (FIG. **3**) and expressed markers consistent with the canonical tissue-resident memory phenotypes. Lung-resident memory CD4 T cells are primarily identified by CD69 expression following infection or vaccination. While we observed $CD69^{+}CD103^{-}CD4$ Trm subset within the lungs of IAV-nanovax vaccinated mice, we unexpectedly observed a small proportion of $CD69^{+}CD103^{+}$ CD4 T cells as well (FIG. **4**A, **4**C). Although this $CD4^{+}CD69^{+}CD103^{+}$ resident memory phenotype has not been well characterized, a study has reported this subset within the skin. What role these $CD69^{+}$ $CD103^{+}$ CD4 T cells may play in protection against subsequent IAV infections remains to be determined.

Previous studies have shown that the maintenance of Trm T cells within lung niches is influenced by the presence and longevity of antigen depots. Following IAV-nanovax vaccination, we observed the presence of both CD4 and CD8 Trm cells within the lungs on day 32 and 45 post vaccination at numbers similar to those observed in an IAV infected lung (FIG. **4**). Preliminary studies also suggest that CD4 and CD8 Trm responses are present in the lungs out to at least day 100 (FIG. **18**). Our prior studies have shown nanoparticles persist within the lungs for ≥14 days and the continual release of antigen from nanoparticles placed into other tissues ≥30 days following vaccination. Overall this suggests that IAV-nanovax may act as an antigen depot, similar to what is observed during IAV infections, and that this may contribute to the upkeep of lung-resident memory T cells.

In conclusion, we have shown that an i.n. inoculation with a polyanhydride nanovaccine encapsulating IAV proteins (IAV-nanovax) provides protection against homologous and heterologous IAV infections. This protection was associated with the induction of GC B cells in the lungs, robust IAV-specific antibody responses both systemically and locally, and IAV-specific CD4 and CD8 T cell responses within the lungs. Further, this report demonstrates for the first time that i.n. vaccination with polyanhydride nanoparticles can induce tissue-resident memory CD4 and CD8 T cells, confer protection against a heterologous virus challenge, and protect against infection in outbred populations. Altogether these findings highlight the potential of utilizing this nanovaccine platform for vaccine delivery in order to induce both systemic and localized adaptive immunity and provide protection against IAV infections.

Example 2. IAV-Nanovax Induces Influenza Specific Memory CD4 and CD8 T Cell Response in the Nasal Passage and Lungs

FIG. **12** and FIG. **13** illustrate the presence of tissue-resident antigen CD4 T cells, CD8 T cells 45 days post initial vaccination (i.n. challenge with the IAV-nanovax) using surface marker expression algorithms and tissue localization techniques. Within the nasal tissue-resident antigen-reactive T cell population, surface marker expression further documented the presence of memory CD4 T cells and CD8 T cells. Multiple reports in the literature underscore the need for tissue resident memory T cells (referred to as Trm) to establish long-term protection against influenza infection.

The same surface marker expression analysis has been used to document the presence of tissue-resident antigen-reactive CD4 T cells and CD8 T cells in the nasal passages 45 days after initial i.n. challenge with the IAV-nanovax. Within the nasal tissue-resident antigen-reactive T cell population, surface marker expression clearly documented the presence of memory T cells.

Example 3. IAV-Nanovax Administration Induces Both a Local (i.e., Lung) as Well as Systemic (Spleen) Immune Response but that i.n. Administration is Necessary to Drive a Robust Adaptive Immune Response within the Lungs

FIG. **14** illustrates the presence of antigen reactive B cells (germinal center B cells), CD4 T cells and CD8 T cells 45 days after either i.n. or subcutaneous (s.c.) administration of the IAV-nanovax, these antigen reactive cells were only found in the lung after i.n. challenge. Antigen reactive cells were found in the spleen (indicative of system distribution) after both i.n. and s.c. challenge (with more cells found in the spleen after s.c., as expected). The figure further demonstrates that the presence of antigen-reactive B cells, CD4 T cells and CD8 T cells in the spleen after i.n. vaccination with the IAV-nanovax.

Example 4. 2X-Nanovax Confers Protection Against Homologous and Heterologous Viruses Using a Single i.n. Vaccination without a Boost

FIG. **15** and FIG. **16** illustrate the induction of adaptive immunity and protection against influenza challenge (homologous and heterologous viruses) after a single i.n. administration of an IAV-nanovax formulation using a "2X" formulation. Specifically, particles were loaded using 2.5× protein payload (although the same amount of CpG adjuvant was used). This data demonstrates that a single dose may provide systemic protection against Influenza rather than the need for a prime-boost.

Example 5. No Immunity is Observed when Mice are Vaccinated with Nanoparticles that Contain the CpG but Lack the Influenza Immunogenic Proteins

FIG. **17** illustrates that the influenza immunogenic protein payload is necessary to confer protection. Specifically, nanoparticles loaded with influenza target proteins+the CpG adjuvant confer protection whereas nanoparticles loaded with the CpG adjuvant alone do not.

Example 6. Tissue Resident Memory CD4 and CD8 T Cells are Sustained in the Lungs to at Least 100 Days Post IAV-Nanovax Administration

FIG. **18** illustrates the presence of influenza-protective immune T cells in the lung and, importantly, confers protection (FIG. **6**) against both homologous and heterologous influenza virus challenge extending to 100 day post initial administration of IAV-nanovax (IAV-nanovax given i.n.).

Example 7. IAV-Nanovax Generates Immunity and Confers Protection in Pre-Immune Populations

FIG. **19** and FIG. **20** illustrate the induction of neutralizing Ab and protection in pre-immune outbred ferrets after